Hemostasis

Introduction

This lesson assumes mastery of thrombopoiesis, the cells of the blood, and the general concepts of laboratory interpretation and blood products. This first hemostasis lesson focuses primarily on endothelial injury and primary hemostasis—the formation of the platelet plug. Ultimately, secondary hemostasis will utilize the platelet plug to form a fibrin thrombus. We will engage platelet adhesion, activation, and aggregation (primary hemostasis), then sketch out a simplified version of the clotting cascade (secondary hemostasis). A deep dive of secondary hemostasis, of the clotting cascade, is reserved for the next lesson (Clotting #2: *Thrombophilia)*, which will build on the information gained in this lesson.

Initial Endothelial Injury

When the endothelium is damaged, the endothelial cell making the lining of the vessel is removed. This exposes the basement membrane of the endothelium. Not having an endothelial cell lining the blood vessel means that cells are able to exit the blood vessel. When red blood cells exit the vessel, we call it bleeding. To stop bleeding, we need there to be a clot. The normal process of clotting must therefore begin with endothelial injury.

The endothelial cell is equipped with resources that allow it to identify when something has gone wrong and to act to make it right. The endothelial cell possesses both anticoagulants (which prevent clotting) and procoagulants (which promote clotting).

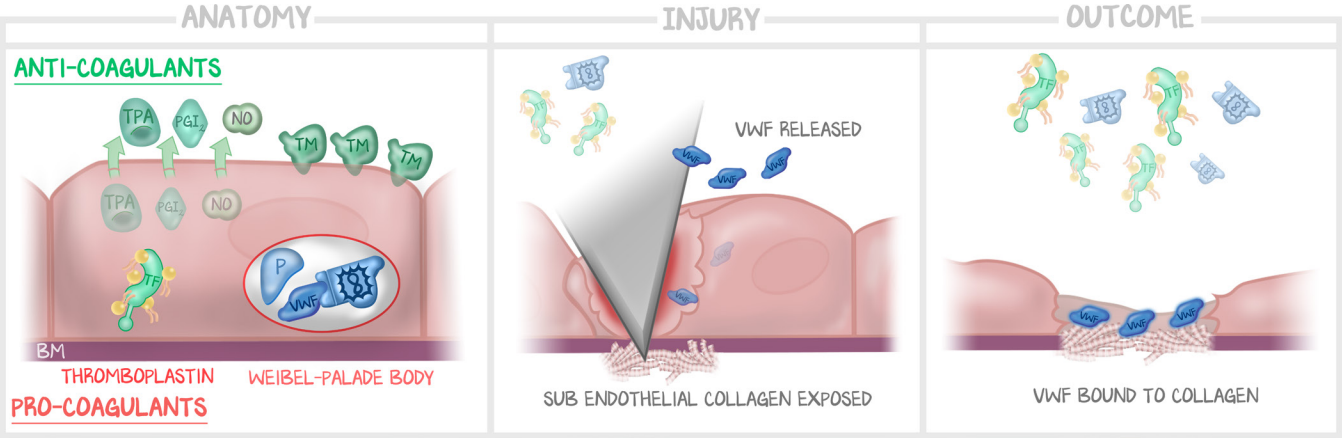

Figure 1.1: Endothelial Cells and Endothelial Cell Injury
An intact endothelial cell expresses anticoagulants to inform the system that no clot is needed. Tissue plasminogen activator (tPA) accelerates fibrin thrombus degradation while prostacyclin (PGI_2) and nitric oxide (NO) induce vasodilation. Thrombomodulin (TM) will accelerate inhibition of the clotting cascade, discussed in the next lesson. At the same time, intact endothelial cells synthesize procoagulants—thromboplastin and Weibel-Palade bodies. Endothelial cell injury exposes subendothelial collagen, impairs anticoagulants, and results in the release of Weibel-Palade bodies and thromboplastin. The end result of endothelial injury is von Willebrand factor (VWF) being bound to collagen and to nearby platelets. Other procoagulants, used later are also released into the capillary lumen with the VWF.

Endothelial cell procoagulants are thromboplastin and Weibel-Palade bodies. **Weibel-Palade bodies** are vesicles that contain von Willebrand factor, factor 8, and P-selectin. Damage to the endothelial cells lining a blood vessel exposes **subendothelial collagen** (the basement membrane) and releases both Weibel-Palade bodies and thromboplastin. The **von Willebrand factor** that is released from the Weibel-Palade bodies binds to that collagen and acts as a tether, connecting the collagen to platelets passing by. **Factor 8** is used in the intrinsic clotting cascade, feed-forwarding the initial platelet response into

the factor response. P-selectin facilitates leukocyte adhesion and is not involved in the clotting process. **Thromboplastin** (also called **tissue factor**) stimulates the extrinsic pathway of the clotting cascade, inducing the formation of a fibrin thrombus independent of the platelet-induced intrinsic pathway we are soon to discuss.

Endothelial cells can also release anticoagulant molecules. Tissue plasminogen activator (discussed later this lesson) reverses clotting. The release of nitric oxide and prostacyclin (PGI_2) to nearby smooth muscle cells results in vasodilation, hastening blood flow to the area. **Thrombomodulin** (caution with the look-alike thromboplastin) is a surface protein on endothelial cells that signals anticoagulation. The idea is that if the endothelial cell is whole, repaired, or otherwise present, they will present proteins and metabolic waste products that signal that the endothelium is whole. When damaged, the absence of these anticoagulants and the release of procoagulants induce clot formation.

Primary Hemostasis = Platelet Plug

Platelets circulate through the bloodstream and form the initial response to endothelial injury by forming the **platelet plug**. Through a coordination of glycoprotein receptors, platelets adhere to each other and to the site of endothelial injury. This forms a mass of platelets over the injured endothelium. This platelet plug was what stopped the bleeding following a surgical incision we discussed in Inflammation and Neoplasia #4: *Wound Healing*. Now we're going to go through the details of how that plug forms.

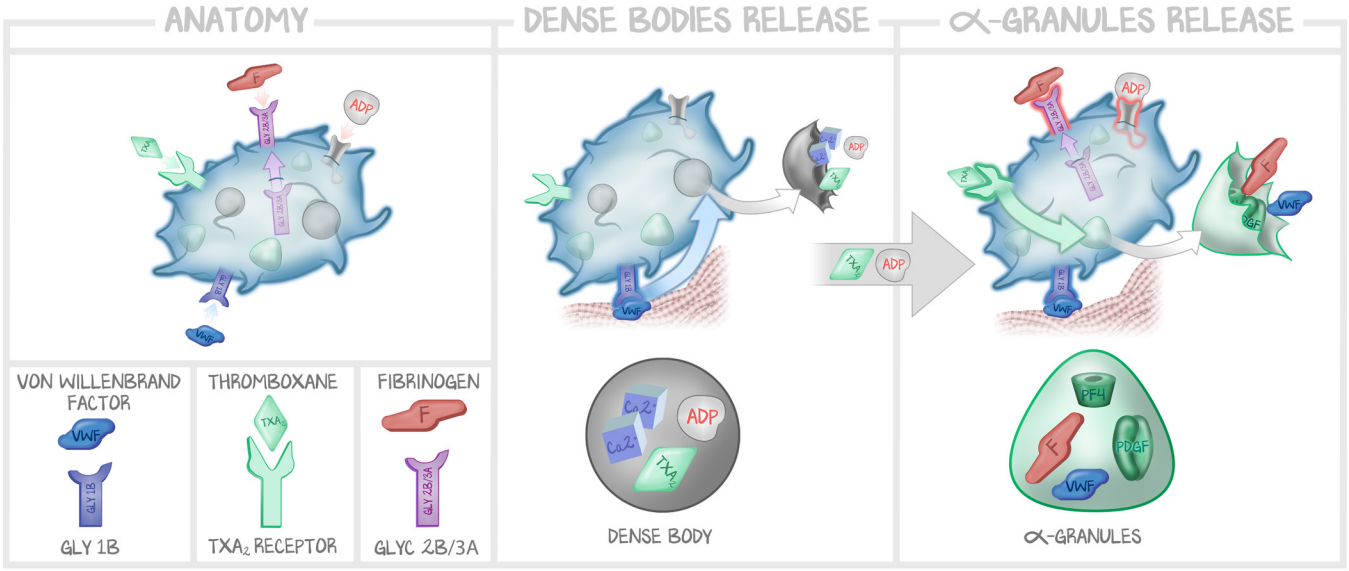

Figure 1.2: Platelet Anatomy
Platelets, despite having no nucleus or mitochondria, are quite complex. They express glycoprotein 1b, which acts as a receptor for von Willebrand factor. The binding of VWF to glycoprotein 1b induces the release of dense bodies. Dense bodies contain ADP, TXA_2, and calcium. The activation of TXA_2 receptors induces the release of α-granules. α-granules contain fibrinogen and von Willebrand factor, amongst other molecules. The binding of ADP to ADP receptors induces the translocation of glycoprotein 2b/3a.

Endothelial cells release **von Willebrand factor** into the lumen of the vessel, which bind to **subendothelial collagen**, the exposed extracellular matrix of the endothelial cell basement membrane. VWF is a protein attached to collagen. **Glycoprotein 1b** (gly1b) is a receptor within the plasma membrane of platelets. VWF is the ligand for the glycoprotein 1b receptor. Platelets passing by bind their glycoprotein 1b to the VWF attached to the subendothelial collagen. This process of a platelet

using glycoprotein 1b to bind to VWF, effectively arresting the platelet at the site of endothelial injury (tethered to the collagen by VWF and glycoprotein 1b) is called **adhesion.** Not many platelets will be near enough to the collagen or VWF to adhere. Even still, that small number is enough to release the signal for the rest to join. The binding of VWF to gly1b keeps the platelets at the site of injury. It also induces the release of dense bodies and α-granules from those tethered-by-VWF platelets.

Dense bodies are cytoplasmic vesicles within platelets that contain thromboxane A_2 (**TXA_2**), adenosine diphosphate (**ADP**), and calcium (**Ca^{2+}**). TXA_2 and ADP are released as ligands near the site of adhered platelets. Platelets passing by have receptors on their plasma membrane for TXA_2 and for ADP (specifically, ADP-$P2Y_{12}$ receptors). The adhered platelets release ligands that bind to and activate receptors on other platelets not yet adhered to the site of injury. This in turn activates the platelet. Platelet **activation** is defined by the binding of ADP to the ADP-$P2Y_{12}$ receptor, which then translocates the cytoplasmic **glycoprotein 2b/3a** into the plasma membrane.

The **α-granules** contain additional VWF (which facilitates more platelet adhesion via gly1b to the subendothelial collagen) and **fibrinogen**. There is also platelet-derived growth factor and platelet factor 4, which are relevant to other disease processes, but not relevant to clotting, so will not be discussed further. **Fibrinogen** acts as the ligand for glycoprotein 2b/3a, through which two platelets can be connected. The glycoprotein 2b/3a receptor of one platelet is bound to the fibrinogen, while the fibrinogen is bound to the glycoprotein 2b/3a of a neighboring platelet. The activation of platelets results in many glycoprotein 2b/3a receptors being expressed, so many platelets end up connected to each other. Activated platelets (both those that adhered to VWF and those activated passing by) begin clustering together, linked to one another by gly2b/3a-fibrinogen, the mass of them tethered to the subendothelial collagen by VWF-attached-to-gly1b of adhered platelets. This accumulation of platelets connected by fibrinogen is called **aggregation**.

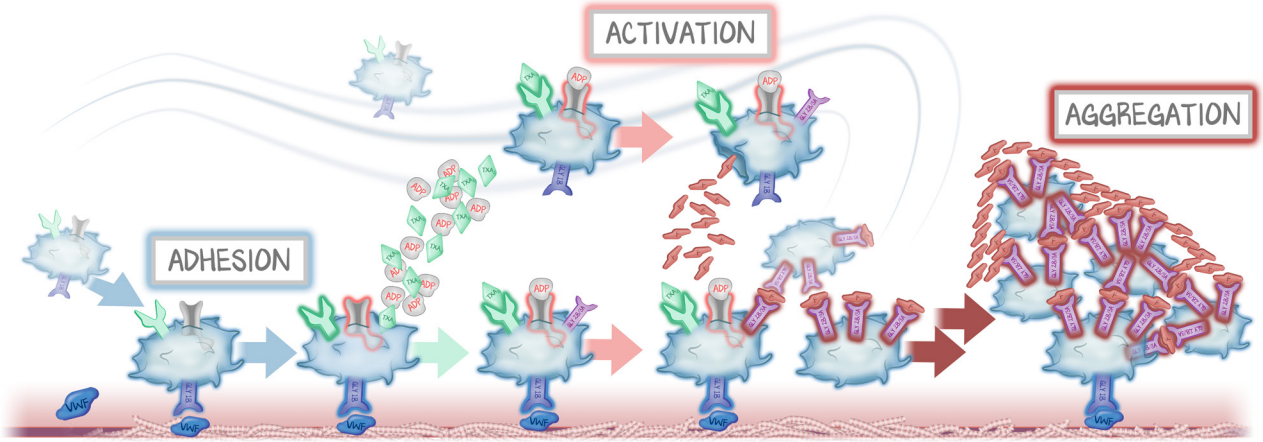

Figure 1.3: Steps in Primary Hemostasis
A small number of platelets bind glycoprotein 1b to VWF. This is adhesion. These platelets release dense bodies. The TXA_2 and ADP released from these dense bodies binds to receptors on passing by platelets and to receptors on the adhered platelet. All platelets stimulated by TXA_2 and ADP receptor stimulation release α-granules and express glycoprotein 2b/3a. This is activation. The glycoprotein 2b/3a of all platelets binds to other platelets' glycoprotein 2b/3a via fibrinogen. This is aggregation.

At the end of primary hemostasis is a mass of platelets, linked to each other by **fibrinogen** (fibrin-ogen), called a **platelet plug**. This initial formation of the platelet plug prevents the escape of red blood cells from the lumen (stops bleeding) and provides the substrate for secondary hemostasis. The platelet plug is not sturdy, is soluble, and can easily tear free from the endothelial damage, necessitating a more permanent solution. Enter secondary hemostasis.

Secondary Hemostasis Is Factor Clotting

The clotting cascade is intimidating for everyone who first happens upon it. It is so intimidating because there are thirteen factors, and each can exist in the inactive or active form. At first glance, twenty-six components to a metabolic pathway just isn't worth it to try. But if we simplify it down some, it becomes more manageable. First, Arabic numerals only, no Roman numerals. Second, no 9 and 9a, only factor 9 and activated factor 9. Third, a memory tool for keeping the clotting cascade straight, as follows.

Here's a simple way to remember what factors do what. The **common pathway** can be recalled by the phrase, "1 five in 5, 2 fives in 10." The common pathway is factors 1, 2, 5, and 10. The **extrinsic pathway** is **exclusive**, so has only one clotting factor, factor 7. The **intrinsic pathway** is, "everyone else up to 12." If you put them near one another, as in Figure 1.4, you may notice that the "top" of the clotting cascade is factors 7–12, with 10 out of place. That means, since 7 is extrinsic, 10 is common, that the rest, 8, 9, 11, and 12, are the intrinsic pathways.

What about factors 3, 4, 6, and 13? Learn that they do not exist. There is no disease state, therapeutic intervention, or regulatory step that involves them. "*One five in 5, two fives in 10; 7 on its own; everyone else up to 12.*"

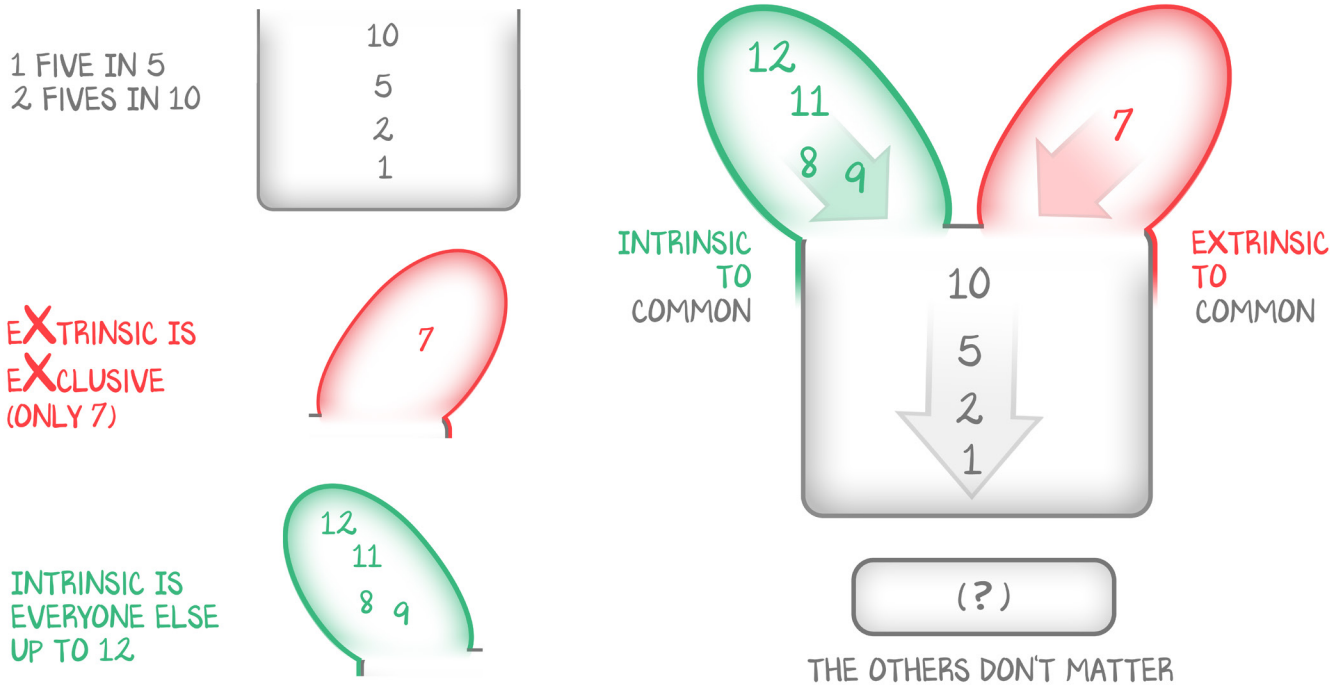

Figure 1.4: The Clotting Cascade, Simplified
This organizer is a method for remembering which factors are in which pathway. The details of activation and regulation are covered in the next lesson.

Now let's go a little deeper.

The **intrinsic pathway** is the pathway initiated by **platelets**. Factors activate each other, one after the other. **Activated factor 8 activates factor 9**. The beginning of the intrinsic pathway is platelet aggregation. The end of the intrinsic pathway is activation of factor 9. Activated factor 9 then activates factor 10 of the common pathway.

The **extrinsic pathway** is the pathway initiated by the **endothelial cells**. Endothelial cells, in addition to the Weibel-Palade bodies (which have factor 8 in them), also release **thromboplastin**. Thromboplastin, also called tissue factor, activates the only factor of the extrinsic pathway, factor 7. The beginning of the extrinsic pathway is endothelial damage. The end of the extrinsic pathway is **activated factor 7**. Activated factor 7 then activates factor 10 of the common pathway.

The **common pathway** is triggered either by the intrinsic pathway (factor 9) or by the extrinsic pathway (factor 7). It always starts with the **activation of factor 10**. Factor 10 then **activates factor 2** using **active factor 5** as a cofactor. Activated factor 2 then **activates factor 1**. "Activation" throughout this discussion has meant "proteolytic cleavage." An inactive clotting factor precursor has some amino acids cleaved away, which results in an activated clotting factor. Factor 2 is (pro)thrombin; the pro- is cleaved away to reveal activated factor 2, **thrombin**. Thrombin activates factor 1, fibrin(ogen), by cleaving the -ogen away, revealing the activated factor 1, **fibrin**. Thrombin also activates factor 8 (stimulating the intrinsic pathway) and factor 5 (stimulating the common pathway). Thrombin also stimulates the formation of a **fibrin thrombus** (fibrin monomer substrates, activated by thrombin) using **calcium** as a cofactor.

Calcium? Fibrinogen? Where did we see those things before? In primary hemostasis. The formation of the platelet plug ended with platelets aggregated together by fibrinogen. The dense bodies release calcium. Both endothelial damage and platelet activation result in the initiation of a clot, a fibrin thrombus.

These steps are reviewed in the next lesson. They are taught again in the context of the regulation of the system and laboratories that monitor them.

Anti-Clot

If there were only this feedforward mechanism, all there would be in response to endothelial injury is more clotting. More clotting would eventually fill the lumen, obstructing blood flow. This is a pathologic state called a **deep vein thrombosis** (a blood clot in the veins) or an **arterial thrombosis** (a blood clot in the artery). Both cause problems for blood flow. Since that doesn't happen to most people, and is a disease state when it does, something must be around to turn off the clotting, to remove the fibrin thrombus as the endothelium heals.

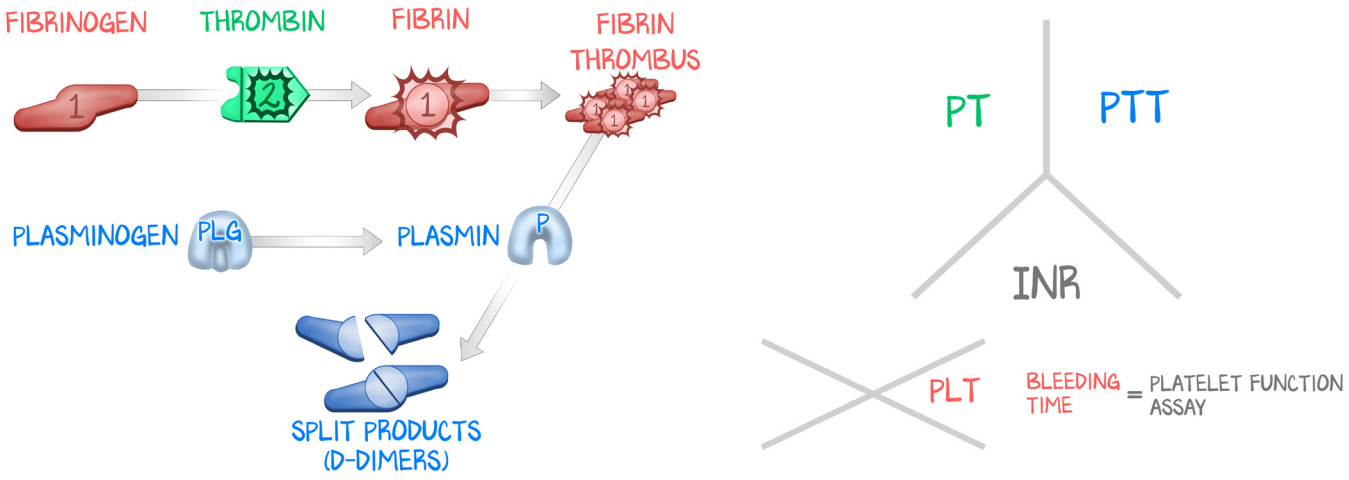

Figure 1.5: Anti-Clot and Clotting Labs
Fibrinogen is activated by thrombin to fibrin. Thrombin also takes the fibrin monomers and turns them into a fibrin polymer, forming the final fibrin thrombus. Plasminogen is activated by tissue plasminogen activator to plasmin. Plasmin degrades the fibrin polymer into split products—into protein that is not simply fibrin monomers, but useless protein conglomerates that must be degraded for later. A decreased fibrinogen and elevated split products is evidence that clotting is happening. The PT and PTT are used to assess adequacy of the clotting cascade. Platelet count and functional assay assess primary hemostasis.

Plasminogen is activated by proteolytic cleavage to **plasmin** by the enzyme tissue plasminogen activator (tPA). The fibrin thrombus is a mesh of cross-linked fibrin monomers. Plasmin chews up (proteolytic cleavage) those cross-linked fibrin monomers and releases them as **split products** (also called **D-dimers**).

Clotting Labs

The **PT** measures the effects of the **extrinsic pathway**. The PT can be represented as the INR; PT and INR mean the same thing. They are used as a method for tracking the anticoagulation status of a patient on warfarin (discussed in Clotting #3: *Clotting Pharmacology*). The **PTT** measures the intrinsic pathway and is used to track the response to unfractionated heparin. Right now, these are facts that need to be memorized. Through subsequent lessons we will engage them over and over again, so that PT = extrinsic = warfarin and PTT = intrinsic = heparin will be intuitive. The **common pathway** is a product of both pathways; defects of the common pathway may reveal an elevation in both the PT and the PTT.

The **complete blood count** includes a **platelet count**. If there is a platelet dysfunction, it can be because there aren't enough platelets (informed by the platelet count) or because platelet function is impaired. While a platelet count can tell you that the problem is from platelet number, a **platelet functional assay** can ascertain whether the problem is functional. A **bleeding time**, though not commonly reported in clinical practice, is used often within the basic sciences as an indication of platelet dysfunction.